Synthesis and Antitumor Activity of Novel 6-Alkyl-6-demethylmitomycins

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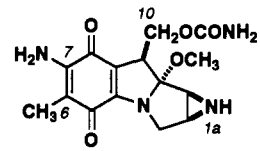
A series of 6-alkyl-6-demethylmitomycins (**1**–**5**) was synthesized and evaluated for anticellular and antitumor activities. These novel compounds were prepared by Michael addition of various carbanion species to 6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-methylidenemitosane (**6** and **9**) followed by treatment with NH_3 or $MeOH/K_2CO_3$. Alkylation at the C-6 position of 6-demethyl-6-selenide (**7**) was also useful for the alternative synthesis of 6-alkyl-6-demethylmitomycins. The antitumor activity of these derivatives was evalulated, and 6-demethyl-6-ethylmitomycin A (**2a**) was found to exhibit excellent activity against S-180 solid tumor in mice. The structure–activity relationship is also discussed.

Introduction

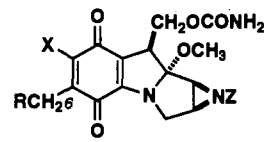
Mitomycins are well known to be potent antitumor antibiotics, produced by various *Streptomyces* cultures.[1] Among these compounds, mitomycin C (MMC) has been extensively used in cancer chemotherapy against a variety of solid tumors but also has detrimental side effects such as severe bone marrow suppression and gastrointestinal damage. In the hope to find mitomycin candidates of the next generation, about a thousand derivatives have been synthesized.[2] Concurrently, some studies have also indicated that the antitumor activity of a derivative is notably influenced by physicochemical factors, *e.g.*, lipophilicity of the molecule and reduction potential of the quinone moiety.[3] During the course of our study of mitomycin chemistry, we have reported in previous papers, including the preceding one, the synthesis of C-6-methyl-labeled compounds and several derivatives by means of the functionalization at the C-6-methyl position via the nucleophilic introduction of functional groups to 6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-methylidenemitosane (**6** and **9**)[4] or at the C-6 position via the electrophilic introduction of functional groups to 6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)mitosane (**7**).[5] By use of these strategies, introduction of various alkyl groups to the C-6 position intended to control lipophilicity is potentially possible. Herein, we describe the synthesis of these 6-alkyl-6-demethylmitomycin derivatives and their antitumor activities.

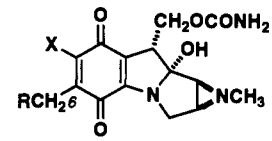
Chemistry

In view of the effectiveness of 6-methylidene intermediates as a Michael acceptor, we reacted **6**,[4] prepared by the method described in the preceding paper, with Me_2CuLi (Schemes 1 and 2). The Michael addition proceeded successfully at −78 °C and afforded an adduct, **10a**, in 51% yield. However, since the enone **6** is highly reactive, undesirable dimeric byproducts[6] were formed during the reaction and formation of **10a** was suppressed. Therefore, the reaction of **6** with Me_2CuLi in the presence of phenol



Mitomycin C
(MMC)





1: MMC type (X = NH_2, Z = H)
2: MMA type (X = CH_3O, Z = H)
3: PFM type (X = NH_2, Z = CH_3)

4: MMD type (X = NH_2)
5: MMB type (X = CH_3O)

Figure 1. Structure of mitomycin C and 6-alkyl-6-demethylmitomycins.

was attempted in order to quench the highly reactive enolic intermediate formed in situ.[7] As a result, the yield of **10a** was slightly improved (57%). Similar reactions using various R_2CuLi were performed, and compounds **10** were obtained in reasonable yields. Grignard reagents were also applicable for the reaction with **6** in the presence of catalytic amounts of CuI. However, when n-$C_{11}H_{23}MgBr$ or n-$C_{19}H_{39}MgBr$ was reacted with **6**, reactions were performed only under prolonged reaction at the elevated temperature (−20 °C) and the yields of adducts were somewhat lower.[8] The same procedures as those described above could be applied to isolated crude **9**,[4c] having the MMB skeleton, for the synthesis of adduct **13**. Nitromethane was also used as a soft nucleophile, and an adduct, **10h**, was formed by the reaction with **6** in the presence of a base.

Conversions of these 7,7-ethylenedioxy intermediates into mitomycin derivatives were achieved by the method described previously.[4a] For the synthesis of **1** and **4** having the MMC and MMD skeletons, treatment of the adducts **10** and **13** with NH_3 in MeOH at room temperature was employed, whereas treatment of the adducts with K_2CO_3 in MeOH at room temperature afforded **2** and **5** having the MMA and MMB skeletons, respectively. For the synthesis of **3** having the porfiromycin (PFM) skeleton, 1a-methylation of **1** using MeI was employed.

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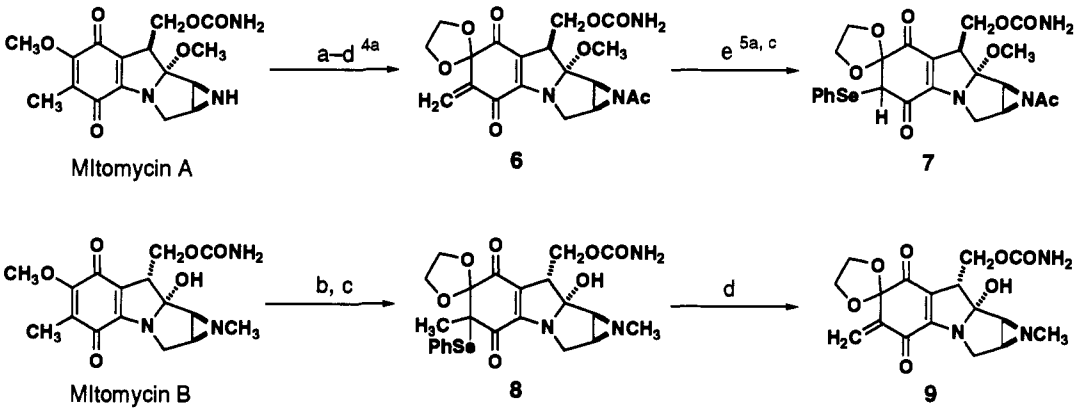
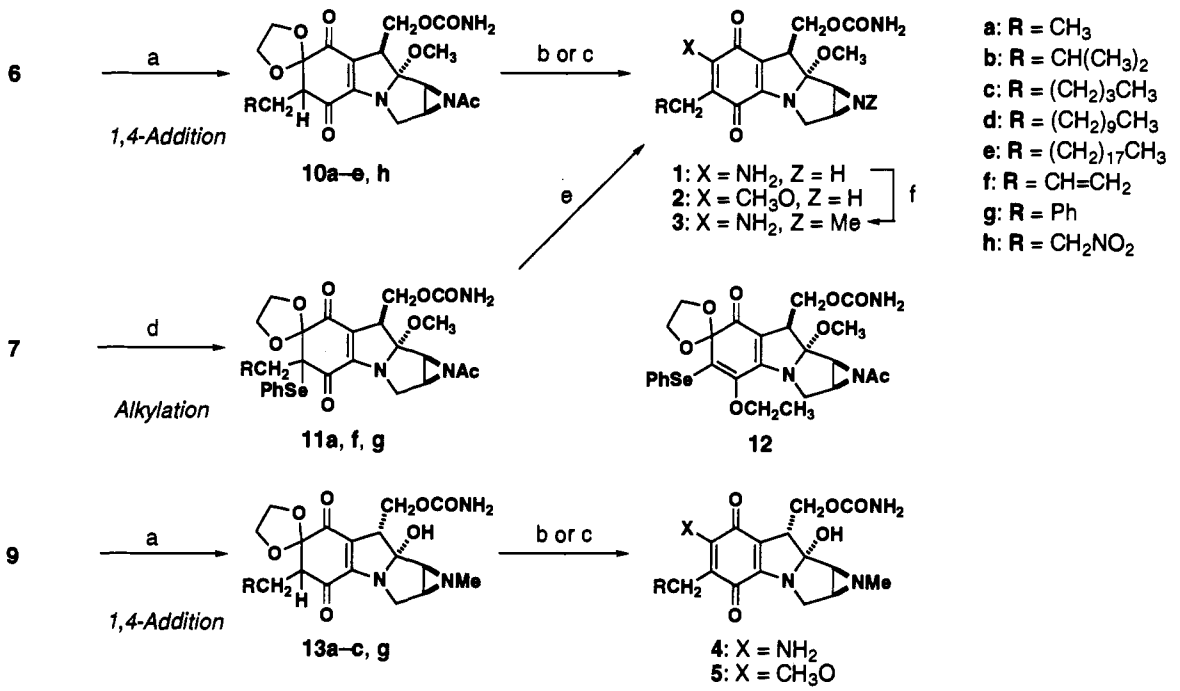
Scheme 1[a]



[a] (a) Ac_2O, pyridine; (b) $HO(CH_2)_2OH$, KOH; (c) PhSeBr, NEt_3; (d) mCPBA, K_2CO_3, −40 °C to room temperature; (e) *N*-(phenylseleno)morpholine, H^+.

Scheme 2[a]



[a] (a) R_2CuLi or RMgX, CuI or $MeNO_2$, K_2CO_3; (b) NH_3, MeOH; (c) K_2CO_3, MeOH; (d) RCH_2I, K_2CO_3 or RCH_2I, Cs_2CO_3; (e) NH_3, dimedone, MeOH; (f) MeI, K_2CO_3.

Alkylation of 7[5] with alkyl halide seems to be effective for the alternative preparation of 6-alkyl-6-demethylmitomycins (Schemes 1 and 2). As a result, compound 7 was treated with allyl bromide in the presence of K_2CO_3 to afford an adduct, **11f**, in 35% yield. Similar results were obtained by the reaction with benzyl bromide. However, in the case of ethyl iodide, ethyl enol ether **12** was obtained predominantly. To avoid undesirable *O*-alkylation, Cs_2CO_3 was used as a base, and consequently, the desired product **11a** was obtained preferentially. The yield of **11f** was also increased 50%, based on 7, by the reaction using Cs_2CO_3 instead of K_2CO_3. The deseleneylation of 11 and its conversion into 1 having the MMC skeleton was achieved by treatment with NH_3 in MeOH in the presence of dimedone.[9] This one-pot conversion was also applicable to the mixture of 11 and 12.[10] These results are summarized in Table 1.

Biological Activity and Discussion

Table 2 shows in vitro anticellular activity against HeLa S_3 cells and in vivo antitumor activity against sarcoma 180 solid tumor in mice. Many derivatives were found to show effective in vitro IC_{50} values in comparison to that of MMC (**1h**, **2a–d,g**, and **5a,g**). There is a marked tendency for the 6-alkyl-6-demethyl-7-methoxymitosanes to have increased in vitro activity compared to the 6-alkyl-7-amino-6-demethylmitosanes, which is also observed in naturally occurring original mitomycins.[11] Above all, the effect of the C-6 substituents of the derivatives seems not to contribute to an increase of in vitro activity. Such a pattern was clearly observed in **2d,e**. On the other hand, the in vivo activity (*T/C*) of most derivatives is lower than that of MMC even at higher doses, and only 6-demethyl-6-ethylmitomycin A (**2a**) showed excellent activity. Compounds **2c,g** and **5a** also displayed moderate activity. These results suggest that the optimal lipophilicity of steric effect caused by the C-6 substituent does exist and that the lower alkyl group such as ethyl is suitable for the C-6 substituent.

In conclusion, various mitomycin derivatives having several substituents at the C-6 position were synthesized and evalulated for their antitumor activity in vitro and in vivo. Several compounds, particularly 7-methoxy derivatives, showed higher in vitro activity than MMC; however, this did not reflect on in vivo activity. Nevertheless, among

Table 1. Preparations of 6-Alkyl-6-demethylmitomycins

compd	CH_2R	MM skeleton	substr	conditions[a] step 1	conditions[a] step 2	yield (%)[b] step 1	yield (%)[b] step 2	empirical formula[c]	^1H NMR (δ, ppm)[d] C-6 subst
1a	CH_2CH_3	MMC	6	a_1	b	51	58	$C_{16}H_{20}N_4O_5 \cdot 0.5CHCl_3$	1.08 (t, J = 7.4 Hz, 3 H), 2.61 (q, J = 7.4 Hz, 2 H)
			6	a_2	b	57	56		
			7	d_2	e	overall 17			
1b	$CH_2CH(CH_3)_2$	MMC	6	a_3	b	28	73	$C_{18}H_{24}N_4O_5 \cdot 0.3H_2O$	0.91 (d, J = 6.4 Hz, 6 H), 1.95–2.16 (m, 1 H), 2.51 (d, J = 7.4 Hz, 2 H)
1c	$(CH_2)_4CH_3$	MMC	6	a_1	b	35	54	$C_{19}H_{26}N_4O_5$	0.89 (t, J = 6.9 Hz, 3 H), 1.26–1.40 (m, 6 H), 2.20–2.24 (m, 2 H)
1d	$(CH_2)_{10}CH_3$	MMC	6	a_3	b	overall 1.0[e]		$C_{25}H_{38}N_4O_5$[f]	0.86 (t, J = 6.6 Hz, 3 H), 1.1–1.4 (m, 16 H), 1.56 (m, 2 H), 2.63 (m, 2 H)
1e	$(CH_2)_{18}CH_3$	MMC	6	a_3	b	overall 1.9[e]		$C_{33}H_{54}N_4O_5$[f]	0.87 (t, J = 6.5 Hz, 3 H), 1.1–1.5 (m, 32 H), 1.56 (m, 2 H), 2.63 (m, 2 H)
1f	$CH_2CH{=}CH_2$	MMC	7	d_2	e	50	46	$C_{17}H_{20}N_4O_5 \cdot 0.3CHCl_3$	3.3–3.5 (m, 2 H), 4.99 (dd, J = 1.5, 9.9 Hz, 1 H), 5.21 (dd, J = 1.7, 19.0 Hz, 1 H), 5.85–6.02 (m, 1 H)
1g	CH_2Ph	MMC	7	d_1	e	overall 22		$C_{21}H_{22}N_4O_5$	3.96 (d, J = 15.4 Hz, 1 H), 4.04 (d, J = 15.4 Hz, 1 H), 7.08–7.26 (m, 3 H), 7.41–7.57 (m, 2 H)
1h	$(CH_2)_2NO_2$	MMC	6	a_4	b	overall 29		$C_{16}H_{19}N_5O_7$[f]	3.27–3.40 (m, 2 H), 4.70 (t, J = 7.6 Hz, 2 H)
2a	CH_2CH_3	MMA	6	a_2	c	57	60	$C_{17}H_{21}N_3O_6$	0.98 (t, J = 7.2 Hz, 3 H), 2.40 (q, J = 7.2 Hz, 2 H)
2b	$CH_2CH(CH_3)_2$	MMA	6	a_3	c	28	65	$C_{19}H_{25}N_3O_6$[f]	0.86 (d, J = 6.4 Hz, 6 H), 1.85 (m, 1 H), 2.35 (m, 2 H)
2c	$(CH_2)_4CH_3$	MMA	6	a_1	c	35	75	$C_{20}H_{27}N_3O_6$	0.89 (br t, J = 6.9 Hz, 3 H), 1.23–1.43 (m, 6 H), 2.22–2.41 (m, 2 H)
2d	$(CH_2)_{10}CH_3$	MMA	6	a_3	c	overall 16[e]		$C_{26}H_{39}N_3O_6$[f]	0.87 (br t, J = 6.7 Hz, 3 H), 1.0–1.4 (m, 16 H), 1.43 (m, 2 H), 2.45 (m, 2 H)
2e	$(CH_2)_{18}CH_3$	MMA	6	a_3	c	overall 10[e]		$C_{34}H_{55}N_3O_6$[f]	0.87 (t, J = 6.7 Hz, 3 H), 1.2–1.4 (m, 32 H), 1.4–1.5 (m, 2 H), 2.47 (br t, J = 6.6 Hz, 2 H)
2g	CH_2Ph	MMA	6	a_2	c	58	49	$C_{22}H_{23}N_3O_6 \cdot 0.2H_2O$	3.75 (d, J = 13.7 Hz, 1 H), 3.81 (d, J = 13.7 Hz, 1 H), 7.15–7.35 (m, 3 H), 7.40–7.45 (m, 2 H)
3a	CH_2CH_3	PFM	1a	f		58[g]		$C_{17}H_{22}N_4O_5 \cdot 1.0H_2O$	1.09 (t, J = 7.5 Hz, 3 H), 2.63 (q, J = 7.5 Hz, 2 H)
4a	CH_2CH_3	MMD	9	a_1	b	40[h]	25	$C_{16}H_{20}N_4O_5$	1.04 (t, J = 7.5 Hz, 3 H), 2.57 (q, J = 7.5 Hz, 2 H)
5a	CH_2CH_3	MMB	9	a_1	c	40[h]	41	$C_{17}H_{21}N_3O_6 \cdot 0.6H_2O$	0.92 (t, J = 7.7 Hz, 3 H), 2.3–2.4 (m, 2 H)
5b	$CH_2CH(CH_3)_2$	MMB	9	a_3	c	30[h]	42[e]	$C_{19}H_{25}N_3O_6$[f]	0.82 (d, J = 6.9 Hz, 6 H), 1.78 (m, 1 H), 2.28 (m, 2 H)
5c	$(CH_2)_4CH_3$	MMB	9	a_2	c	30[h]	63	$C_{20}H_{27}N_3O_6 \cdot 0.3H_2O$	0.80 (br t, J = 6.9 Hz, 3 H), 1.1–1.4 (m, 6 H), 2.3–2.4 (m, 2 H)
5g	CH_2Ph	MMB	9	a_2	c	21[h]	82	$C_{22}H_{23}N_3O_6$	3.71 (s, 2 H), 7.10–7.36 (m, 5 H)

[a] Reaction conditions: a_1, R_2CuLi; a_2, R_2CuLi, PhOH; a_3, RMgX, CuI; a_4, $MeNO_2$, K_2CO_3; b, NH_3, MeOH; c, MeOH, K_2CO_3; d_1, RCH_2X, K_2CO_3; d_2, RCH_2X, Cs_2CO_3; e, NH_3, dimedone, MeOH; f, MeI, K_2CO_3. [b] Unless otherwise noted, yields are indicated for each step. [c] Determined by elemental analysis unless otherwise noted. Analytical results were within ±0.40% of theoretical values. [d] The solvent was pyridine-d_5 except for 1c and 2c ($CDCl_3$). [e] 10-*O*-Decarbamoylmitomycin was obtained as a byproduct. [f] Determined by FAB-HRMS. Analytical results were within ±5 mmu of the theoretical value. [g] Yield based on 1a. [h] Yield based on 8.

the derivatives, 6-demethyl-6-ethylmitomycin A (**2a**) exhibited excellent in vitro and in vivo activity. It is one of the candiates for the next generation of mitomycin derivatives.

Experimental Section

Unless otherwise noted, materials were obtained from commercial suppliers except for mitomycins and were used without purification. THF was distilled from sodium/benzophenone immediately prior to use. Benzene was distilled at atmospheric pressure and stored over 4-Å molecular sieves. Proton nuclear magnetic resonance (^1H NMR) spectra were recorded on Bruker AM 400, JEOL JNM-GX270, and JEOL JNM-EX270 instruments. Mass spectral (MS) data were obtained from Hitachi M-80B and JEOL JMS-D300 mass spectrometers. Infrared spectra (IR) were recorded on a Nihon Bunko IR-810 instrument. Elemental analyses were performed using a Perkin-Elmer 2400 C, H, N analyzer. The purity of the samples was checked by chromatographic methods (HPLC and TLC) and careful analysis of NMR spectra. The representative analytical data are listed in Table 1.

6-Demethyl-6-ethylmitomycin C (1a). (1) Preparation from Enone 6 (Method A). To a stirred solution of **6** (80 mg, 0.19 mmol) in THF (4 mL) was added dropwise Me_2CuLi (0.13 M in THF, 3.0 mL, 2.0 equiv) at −78 °C, and the mixutre was stirred for 30 min at that temperature. The resulting reaction was quenched by the addition of aqueous NH_4Cl solution and the mixture extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated on a rotary evaporator. The residue was purified by column chromatography (silica gel, 97:3 $CHCl_3$/MeOH as an eluent) followed by drying under vacuum to afford an adduct, **10a** (42 mg, 51% based on **6**). The adduct (52 mg, 0.12 mmol) was treated with a MeOH solution of NH_3 (6.8 M, 0.5 mL) in MeOH (5 mL) at room temperature. After 20 h, the volatiles were removed on a rotary evaporator and the residue was purified by column chromatography (silica gel, 20:1 $CHCl_3$/MeOH as an eluent). The paste obtained was triturated with $CHCl_3$–*n*-hexane followed by drying under vacuum to afford **1a** (24 mg, 58%) as a purple powder.

(2) Preparation from Enone 6 (Method B). To a stirred solution of **6** (422 mg, 1.01 mmol) and PhOH (0.96 g, 10 mmol, 10 equiv) in THF (100 mL) was added dropwise Me_2CuLi (0.25 M in THF–*n*-hexane, 20 mL, 5.0 equiv) at −78 °C over a period of 5 min, and the mixture was stirred for an additional 30 min at that temperature. The reaction was quenched by addition of an aqueous NH_4Cl solution, and the mixture was extracted with ethyl acetate. The organic layer was washed with aqueous NH_4Cl solution and brine, dried over Na_2SO_4, and concentrated on a rotary evaporator. The paste obtained was purified by column chromatography (silica gel, 20:1 $CHCl_3$/MeOH as an eluent) followed by trituration with $CHCl_3$–*n*-hexane and drying under vacuum to afford an adduct, **10a** (249 mg, 57% based on **6**), as a reddish purple powder. The same procedure as described in the synthesis of **1a** (method A) was employed to convert **10a** into **1a** (56%).

(3) Preparation from 6-Demethyl-6-selenide (7) (Method C). To a stirred solution of **7** (56 mg, 0.10 mmol) in MeCN (1.0 mL) were added Cs_2CO_3 (65 mg, 0.20 mmol, 2.0 equiv) and C_2H_5I (100 μL, 1.25 mmol, 12.5 equiv). After 45 h at room temperature, the resulting mixture was diluted with CH_2Cl_2 and washed successively with phosphate buffer (pH 4), an aqueous $NaHCO_3$ solution, and brine. The organic layer was dried over Na_2SO_4 and concentrated on a rotary evaporator to afford a mixture of

Table 2. In Vitro and in Vivo Antitumor Activities of 6-Alkyl-6-demethylmitomycins

compd	HeLa S_3[a] IC_{50} (μM)	S-180 (sc–iv)[b] ED_{50}[c] (mg/kg)	S-180 (sc–iv)[b] OD[d] (mg/kg)	S-180 (sc–iv)[b] T/C min.[e]
1a	>10	38	46	0.42
1b	>10		46	0.78
1c	>10	nt[f]	nt[f]	nt[f]
1d	>10	nt	nt	nt
1e	9.0	nt	nt	nt
1f	10	nt	nt	nt
1g	>10	nt	nt	nt
1h	1.5	8.0	15	0.44
2a	0.017	2.8	7.4	0.10
2b	0.20	nt	nt	nt
2c	0.087	19	30	0.37
2d	0.64		20	0.80
2e	7.3		46	0.69
2g	0.063	23	30	0.43
3a	nt[f]		46	1.02
4a	>10		20	0.80
5a	0.30	14	30	0.39
5b	6.1		20	0.71
5c	3.1		20	0.79
5g	0.52	37	46	0.41
mitomycin C	0.59–1.5	1.7–6.2	6.0	0.26–0.44
mitomycin A	0.0024	1.3	1.8	0.20

[a] In vitro anticellular activity against HeLa S_3 cells. The cells were cultured in 96-well plates on day 0 and treated with compounds for 1 h on day 1. The anticellular activity was determined according to the method described previously (see ref 2c). [b] In vivo antitumor activity against sarcoma 180. Sarcoma 180 cells were inoculated sc into the axillary region of ddY mice on day 0. Compounds were administered iv on day 1. [c] Dose that gave 50% inhibition of tumor growth calculated from the dose–response curve. [d] Optimal dose. [e] Treated versus control value of tumor volume. Tumor volume was calculated according to the method described previously (see ref 2c). [f] Not tested.

crude **11a** and **12**. To the mixture were added dimedone (50 mg, 3.6 equiv) and a MeOH solution of NH_3 (6.1 M, 2.0 mL), and the mixture was allowed to stand at room temperature for 3.5 h. After the same treatment as that described in method A, compound **1a** (6.0 mg, 17% based on **7**) was obtained as a purple powder. At the same time, the 1a-deacetyl derivative of **12** (14 mg, 23% based on **7**) was also obtained as a byproduct: ^{1}H NMR (400 MHz, pyridine-d_5) δ 1.08 (t, J = 7.4 Hz, 3 H, 6-CH_2CH_3), 2.10 (br s, 1 H, 1a-H), 2.61 (q, J = 7.4 Hz, 2 H, 6-CH_2), 2.74 (dd, J = 1.7, 4.4 Hz, 1 H, 2-H), 3.13 (d, J = 4.2 Hz, 1 H, 1-H), 3.21 (s, 3 H, 9a-OCH_3), 3.61 (dd, J = 1.7, 12.6 Hz, 1 H, 3α-H), 4.03 (dd, J = 4.2, 11.1 Hz, 1 H, 9-H), 4.58 (d, J = 12.6 Hz, 1 H, 3β-H), 5.08 (br t, J = 11.1 Hz, 1 H, 10-H_a), 5.43 (dd, J = 4.2, 10.3 Hz, 1 H, 10-H_b), 7.60 (br s, 2 H, 10-$OCONH_2$), 7.67 (br s, 2 H, 7-NH_2); EI-MS m/z 348 (M^+); IR (KBr) 3430, 3330, 2930, 1710, 1650, 1600, 1540, 1450, 1340, 1260, 1220, 1070 cm^{-1}. Anal. ($C_{16}H_{20}N_4O_5$·0.5$CHCl_3$) C, H, N.

6-Demethyl-6-ethylmitomycin A (2a). To a MeOH (40 mL) solution of **10a** (372 mg, 0.857 mmol) was added K_2CO_3 (237 mg, 1.71 mmol, 2.0 equiv), and the mixture was stirred at room temperature. After 3 h, brine was added to the mixture and the mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated on a rotary evaporator. The paste obtained was purified by column chromatography (silica gel, 2:1 $CHCl_3$/acetone as an eluent) followed by trituration with $CHCl_3$–n-hexane and drying under vacuum to afford **2a** (187 mg, 60%) as a reddish purple paste.

6-Demethyl-6-ethylporfiromycin (3a). To a solution of **1a** (50 mg, 0.14 mmol) in acetone (3.0 mL) were added K_2CO_3 (19 mg, 0.14 mmol, 1.0 equiv) and MeI (0.5 mL, excess), and the mixture was stirred for 23 h at room temperature. After the volatiles were removed on a rotary evaporator, the residue was purified by column chromatography (silica gel, 30:1 $CHCl_3$/MeOH as an eluent) to afford a paste, which was triturated with $CHCl_3$–n-hexane followed by drying under vacuum to afford **3a** (30 mg, 58%) as a purple powder.

6-Demethyl-6-ethylmitomycin D (4a). To a suspension of **8** (1.45 g, 2.72 mmol) and powdered K_2CO_3 (827 mg, 5.99 mmol) in CH_2Cl_2 (50 mL) was added a solution of mCPBA (about 70% purity, 750 mg, 3.0 mmol) in CH_2Cl_2 (25 mL) over a period of 15 min and the mixture stirred for 1 h at that temperature. After 1.5 h at 0 °C, the mixture was filtered through Celite and the filtrate was concentrated on a rotary evaporator. The obtained residue was triturated with CH_2Cl_2–n-hexane and dried under vacuum to afford crude **9** (1.29 g) as a yellow powder. The material was used for further reactions without purification. Crude **9** (1.185 g) was treated according to a similar procedure to that described in the synthesis of **1a** (method A) with Me_2CuLi (0.25 M in THF–Et_2O, 18 mL, *ca.* 1.5 equiv) in THF (20 mL) to afford the adduct **13a** (375 mg, 40% based on **8**). To a solution of **13a** (350 mg, 0.893 mmol) in MeOH (50 mL) was added NH_3 in MeOH (6.1 M, 10 mL), and the mixture was allowed to stand at room temperature for 9.5 h. After a similar treatment to that described in the synthesis of **1a** (method A), compound **4a** (76 mg, 25%) was obtained as a gray powder.

6-Demethyl-6-ethylmitomycin B (5a). A similar procedure to that described in the synthesis of **2a** was employed to convert **13a** (375 mg, 0.957 mmol) into **5a** (144 mg, 41%) as a purple powder.

6-Demethyl-6-(2-methylpropyl)mitomycin C (1b). To a suspension of **6** (425 mg, 1.01 mmol) and CuI (62 mg, 0.33 mmol, 0.32 equiv) in THF (30 mL) was added a solution of *i*-PrMgBr (0.64 M in THF, 4.5 mL, 2.9 equiv) over a period of 50 min at −78 °C, and the mixture was stirred for 10 min at that temperature. The resulting reaction was quenched by addition of an aqueous NH_4Cl solution and the mixture extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated on a rotary evaporator. The residue was purified by column chromatography (silica gel, 30:1–20:1 $CHCl_3$/MeOH as eluents) followed by trituration with $CHCl_3$–n-hexane and drying under vacuum to afford an adduct, **10b** (132 mg, 28%). A similar procedure to that described in the synthesis of 1a was employed to convert **10b** (48 mg, 0.103 mmol) into **1b** (28 mg, 73%) as a purple powder.

6-Demethyl-6-(2-methylpropyl)mitomycin A (2b). A similar procedure to that described in the synthesis of **2a** was employed to convert **10b** (132 mg, 0.285 mmol) into **2b** (73 mg, 65%) as a reddish purple powder.

6-Demethyl-6-(2-methylpropyl)mitomycin B (5b). Crude **9** (645 mg) was treated according to a similar procedure to that described in the synthesis of **1b** with *i*-PrMgBr (0.67 M in THF, 3.0 mL, *ca.* 1.2 equiv) and CuI (90 mg, 0.47 mmol, *ca.* 0.28 equiv) in THF (50 mL) to afford an adduct, **13b** (171 mg, 30% based on **8**), as a slight yellow powder. The above **13b** (161 mg, 0.384 mg) was treated by a similar procedure as that described in the synthesis of **5a** to afford **5b** (63 mg, 42% based on **13b**) as a purple powder. In addition, the 10-*O*-decarbamoyl derivative of **5b** (10 mg, 8% based on **13b**) was obtained as a byproduct.

6-Demethyl-6-*n*-pentylmitomycin C (1c). Compound **6** (190 mg, 0.453 mmol) was treated according to a similar procedure to that described in the synthesis of **1a** (method A) with *n*-Bu_2CuLi (0.094 M in THF–*n*-hexane, 5.3 mL, 1.1 equiv) in THF (6.0 mL) to afford an adduct, **10c** (74 mg, 35%). A similar procedure to that described in the synthesis of **1a** was employed to convert **10c** (43 mg, 0.090 mmol) into **1c** (19 mg, 54%) as a purple paste.

6-Demethyl-6-*n*-pentylmitomycin A (2c). A similar procedure to that described in the synthesis of **2a** was employed to convert **10c** (95 mg, 0.20 mmol) into **2c** (60 mg, 75%) as a reddish purple powder.

6-Demethyl-6-*n*-pentylmitomycin B (5c). Crude **9** (642 mg) was treated according to a similar procedure to that described in the synthesis of **1a** (method B) with *n*-Bu_2CuLi (0.31 M in THF–Et_2O, 41 mL, *ca.* 7.5 equiv) and PhOH (2.0 g, 21 mmol, *ca.* 12 equiv) in THF (50 mL) to afford an adduct, **13c** (174 mg, 30% based on **8**), as a slight yellow powder. Compound **13c** (165 mg, 0.380 mmol) was treated by a similar procedure to that described in the synthesis of **5a** to afford **5c** (96 mg, 63% based on **13c**) as a purple powder.

6-Demethyl-6-*n*-undecylmitomycin C (1d). To a suspension of **6** (808 mg, 1.93 mmol) and CuI (72 mg, 0.38 mmol, 0.20 equiv) in THF (20 mL) was added dropwise *n*-$C_{10}H_{21}MgBr$ (0.97 M, 6.0 mL, 3.0 equiv) over a period of 50 min at −20 °C under an argon atmosphere. After an additional 30 min at −20–0 °C, the reaction mixture was treated by a similar procedure to that described in the synthesis of **1b** to afford a crude adduct, **10d** (132 mg).

Compound **10d** (132 mg, crude) was treated by a similar procedure to that described in the synthesis of **1a** to afford **1d** (9.1 mg, 1.0% based on **6**) as a purple powder. In addition, the 10-*O*-decarbamoyl derivative of **1d** (17 mg, 17%) was obtained as a byproduct.

6-Demethyl-6-*n*-undecylmitomycin A (2d). A similar procedure to that described in the synthesis of **2a** was employed to convert the crude adduct **10d** (495 mg) into **2d** (159 mg, 16% based on **6**) as a reddish purple paste. In addition, the 10-*O*-decarbamoyl derivative of **2d** (107 mg, 12% based on **6**) was also obtained as a byproduct.

6-Demethyl-6-*n*-nonadecylmitomycin C (1e). To a suspension of **6** (850 mg, 2.03 mmol) and CuI (82 mg, 0.43 mmol, 0.21 equiv) in THF (100 mL) was added dropwise *n*-$C_{18}H_{37}MgBr$ (0.86 M, 8.0 mL, 3.4 equiv) over a period of 30 min at −20 °C under an argon atmosphere. After an additional 20 min at that temperature, the reaction mixture was treated by a similar method to that described in the synthesis of **1a** to afford a crude adduct, **10e** (516 mg). A similar treatment to that described in the synthesis of **1a** was employed to convert **10e** (200 mg, crude) into **1e** (8.6 mg, 1.9% based on **6**) as a purple powder. In addition, the 10-*O*-decarbamoyl derivative of **1e** (77 mg, 18% based on **6**) was also obtained as a byproduct.

6-Demethyl-6-*n*-nonadecylmitomycin A (2e). A similar procedure to that described in the synthesis of **2a** was employed to convert the crude **10e** (508 mg) into **2e** (124 mg, 10% based on **6**) as a purple paste. In addition, a small amount of the 10-*O*-decarbamoyl derivative of **2e** was also obtained as a byproduct.

6-Allyl-6-demethylmitomycin C (1f). Compound **7** (56 mg, 0.10 mmol) was treated according to a similar procedure to that described in the synthesis of **1a** (method C) with allyl iodide (100 μL, 1.09 mmol, 11 equiv) and Cs_2CO_3 (65 mg, 0.20 mmol, 2.0 equiv) in acetone (1.0 mL) to afford an adduct, **11f** (30 mg, 50%). A similar treatment to that described in the synthesis of **1a** (method C) was employed to convert **11f** (24 mg, 0.040 mmol) into **1f** (4.6 mg, 46% based on **7**) as a purple powder.

6-Benzyl-6-demethylmitomycin C (1g). Compound **7** (28 mg, 0.050 mmol) was treated according to a similar procedure to that described in the synthesis of **1a** (method C) with benzyl bromide (60 μL, 0.50 mmol, 10 equiv) and K_2CO_3 (35 mg, 0.25 mmol, 5.0 equiv) in acetone (0.50 mL) to afford a crude adduct, **11g** (15 mg). A similar treatment to that described in the synthesis of **1a** (method C) was employed to convert **11g** (15 mg) into **1g** (4.5 mg, 22% based on **7**) as a purple powder.

6-Benzyl-6-demethylmitomycin A (2g). Compound **6** (845 mg, 2.02 mmol) was treated according to a similar procedure to that described in the synthesis of 1a (method B) with PhOH (2.0 g, 21 mmol, 10 equiv) and Ph_2CuLi (0.34 M in THF, 30 mL, 5.0 equiv) in THF (100 mL) to afford an adduct, **10g** (584 mg, 58% based on **6**). A similar treatment to that described in the synthesis of **2a** was employed to convert **10g** (575 mg, 1.16 mmol) into **2g** (238 mg, 49%) as a reddish purple powder.

6-Benzyl-6-demethylmitomycin B (5g). Crude **9** (963 mg) was treated according to a similar procedure to that described in the synthesis of **1a** (method B) with PhOH (2.3 g, 24 mmol, *ca.* 9.4 equiv) and Ph_2CuLi (0.34 M in THF, 40 mL, *ca.* 5.0 equiv) in THF (100 mL) to afford an adduct, **13g** (208 mg, 21% based on **8**). A similar treatment to that described in the synthesis of **5a** was employed to convert **13g** (195 mg, 0.427 mmol) into **5g** (148 mg, 82% based on **13g**) as a reddish purple powder.

6-Demethyl-6-(2-nitroethyl)mitomycin C (1h). To a solution of **6** (73 mg, 0.18 mmol) in $CHCl_3$ were added nitromethane (0.5 mL) and K_2CO_3 (50 mg, 0.36 mmol, 2.0 equiv), and the mixture was stirred at room temperature. After 2 h, the resulting mixture was treated with phosphate buffer (pH 4) and extracted with $CHCl_3$. The obtained organic layer was washed with brine, dried over Na_2SO_4, and concentrated on a rotary evaporator. The residue was treated with a MeOH solution of NH_3 (6.8 M, 0.3 mL) in MeOH at room temperature. After the volatiles were removed on a rotary evaporator, the residue was purified by column chromatography (silica gel, 97:3 $CHCl_3$/MeOH as an eluent) followed by trituration with $CHCl_3$–*n*-hexane and drying under vacuum to afford **1h** (20 mg, 29% based on **6**) as a purple powder.

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Supplementary Material Available: Complete analytical data (^1H NMR, IR, mass spectrum, elemental analysis) for new compounds except **1a** and ^1H NMR spectra (26 pages). Ordering information is given on any current masthead page.

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